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A Study of the root cells of Lupinus Albus as influenced by potassium iodide

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A STUDY OF THE ROOT CELLS OF LUPINUS ALBUS AS INFLUENCED BY POTASSIUM IODIDE

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BY

ETHEL CLAIRE POND

THESIS

FOR

DEGREE OF BACHELOR OF ARTS

IN

GENERAL SCIENCE

COLLEGE OF SCIENCE

UNIVERSITY OF ILLINOIS

PRESENTED JUNE 1910

1510
P77

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May 31 1910

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IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Arts

in General Science

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A STUDY OF THE CELLS OF THE GROWING POINT OF ROOT OF LUPINUS ALBUS THRU THEIR REACTION TO POTASSIUM IODIDE SOLUTIONS.

The state of knowledge concerning plant cells has now reached a point where little or nothing can be added by simple morphologic study. The parts of the cell have been described and any advance toward determining the function of these parts must be made from the physiological point of view. The manner in which the cell constituents respond to outside stimuli is an index to the real nature of these constituents and to their normal role. A living cell, however, is not a stable thing that reacts mechanically to each stimulation, and as Sachs stated years ago, it is not the same today as yesterday and tomorrow will see a new cell in place of today's. To subject, then, a single cell to a certain stimulus would only determine that in the particular physiologic state examined a cell responded thus and so. An experiment that will determine something of cells in general must be performed on cells of all ages and conditions. For this purpose a meristematic region such as found in the growing point of roots is particularly valuable. Here we can subject cells of all ages, various degrees of differentiation and different conditions of nutrition simultaneously to the same influence. Such regions have been used recently in experimental studies

by Nemec (1, 2, 3, 4) and others and tho little or no attention was paid by them to the different physiologic states of the cells in different regions yet a marked difference in the behavior of the cells in these regions can be clearly made out from the results as recorded.

The study of such a region presents many difficulties, however. Fixing agents and stains must be used whose reaction upon the cell parts is not exactly known. Fischer (1899) has in particular protested against the faith put in such artificial aids. Altho it does not seem that all the appearances in fixed cells are artefacts, still great care should be taken not to accept every new aspect of the cell as real, unless such features recur under similar physiologic conditions. Staining reactions cannot ordinarily be interpreted as having chemical significance for physical factors affect staining considerably.

Such a thing, for instance, as the distribution of chromatin particles on a plastin matrix may give an appearance of much chromatin or little, strong staining reaction or slight, according as it is packed together or spread out.

In addition to these obstacles, the minuteness of many cell parts, together with their transparency places them nearly at the limit of vision even with the highest magnification possible.

For these reasons the effect of an outside agent was tried upon root tip cells in hope that by retarding or inhibiting some processes and accelerating others, some features

of cellular activity might become apparent as morphologic changes that are not so in the balanced condition we call "normal" for the cell. The agent whose affect was thus tested was potassium iodide.

METHOD - The root tips of seedlings of Lupinus albus were used in these experiments. These seedlings were grown in the following manner: The beans were soaked 12 hours, planted in moist sphagnum and germinated in the dark for, from 36 to 48 hours or until the roots were from 1/2 to 2 centimeters in length. The seedlings were then placed on wire gauze with their roots extending thru the meshes of the gauze into water beneath. They were allowed to accustom themselves to this medium for 12 hours. At the end of this period the seedlings were transferred to KI solutions of 1/2, 1, 1 1/2 and 2 percents for periods of 1, 2, 3, 4 and 5 hours. At the end of each period of treatment in each percent 6 roots were killed and fixed at once, 6 after a recovery of 6 hours and 6 after 24 hours growth in water. Two fixing agents were used, corrosive sublimate acetic and Hof's modification of Flemming's chromic acid. The tips were imbedded in parafin and stained in Heidenhain's iron haemotoxylin.

THE NORMAL ROOT TIP CELLS.

In order to judge of changes produced in the cells by the KI it is necessary to have a conception of the normal form of these cells. The cells of the meristematic region of

Lupinus has been described by Georgevitch (1907-1908) but since these descriptions are made with view to study of certain features, alone, it is necessary for me to supplement the descriptions. The cells of Phaseolus which in many respects closely resemble Lupinus have been described by Wager (1904) and I take the liberty to refer to his work for some detail.

Physiologically the root may be divided into three regions (Sachs'87) the formative, stretching and differentiating zones. The cells of these regions are different in appearance and function and I shall describe them separately.

The cells of the formative region are quadrilateral or polygonal in outline, and about equidimentional. They are filled with finely fibrillar or alveolar cytoplasm. The vacuoles are small. The nuclei are large as compared to the cell size.

The resting nucleus is circular in outline with a distinct nuclear membrane. The reticulum is fine and fills only the outer 1/4 to 1/3 of the circle as it appears in cross section. The nucleolus is large and circular in outline, and stains dense black with vacuoles which appear gray or brownish. It is surrounded by a clear hof which is crossed by a few strands of the reticulum. Often a little bud like protrusion is seen on the side of the nucleolus.

ROOT CAP - The root cap cells as they are pushed outward from the formative region retain the same outline as before until they reach the outermost zone where they are flattened probably by the tension upon them. Their cytoplasm becomes more and more vacuolated and reduced in amount until in the outer portion

it quite disappears. The nuclei become somewhat smaller and stain less deeply, the nucleoli usually break up into two or three or more parts and seem to waste away. The hof becomes much smaller even in the first cells of the region.

THE REGION OF ELONGATION - The cells formed by the division of the cells of the formative region lie in parallel rows and the degree of their change from the original cells can be traced back on these rows. The cells of both plerome and periblem become lengthened in direction of the root axis. In the periblem the cells increase in both diameters tho not equally, the nuclei become larger as do the nucleoli but the nucleus does not keep its former proportionate size to the whole cell. The cytoplasm contains small vacuoles.

The cells of the border of the plerome become large and rectangular in shape with large nuclei and nucleoli. Those cells forming the inner portion of the plerome become long and narrow so that in many cases the diameter of the nucleus is nearly equal to the width of the cell. The nuclei do not increase in size as do those of the outer layers. The vacuoles in the cytoplasm increase in size as they do in the periblem.

THE ZONE OF DIFFERENTIATION - This region was not used for observation to any extent for the reason that here the more permanent cells are less affected by external agents. The cells here are much elongated, the vacuoles fill the central portion, and the nucleus lies imbedded in the cytoplasmic layer along one of the walls. The nuclei are smaller and stain less deeply than in the regions previously described.

THE MITOTIC FIGURE - Cells in division occur commonly thruout the formative and stretching zones and rarely in the root caps.

The first sign of approaching division is the thickening of the recticular thread and the appearance of the beginning of the spindle in the shape of dome like structures of fibres at opposite sides of the nucleus. As these kinoplasmic structures become more conical the nuclear membrane disappears and the spireme stands out clearly as a mass of heavy darkly stained threads. I did not find the nucleolus taking any such part in this change as is described by Georgevitch (1908) as taking place in Lupinus augustifolius. The nucleolus was often visible in this stage, and in such cases it appeared much reduced in size but was usually still circular in outline and in no case did it appear to be giving off arms that might form chromosomes. I have observed cells which showed large processes extending out from the nucleolus but in most of such cases I could discover no evidence that such cells were about to divide.

The spindle takes two forms according to the shape of the cell in which it is formed. It may be either barrel shaped with no distinct focus at which the fibres meet at the ends - (as figured by Wager - 1904) or it may have two distinct foci where the fibres converge as sharply as they do in the animal cell. The chromosomes are very small and in the equitorial plate they appear so massed together that no separation can be seen between them. The fibres stain clearly and were it not so minute the figure would be easily studied. At this stage nucleoli are quite commonly visible. In most cases, these do

not lie in contact with the chromosomes as is figured by Wager in Phaseolus but may lie at the poles of the spindle, at one side of but separate from the mass of chromosomes or even quite away from the spindle in a corner of the cell. Nucleoli that persist in this manner, are always much reduced in size, mere remnants of their former states. The separation of the chromosomes and the formation of the daughter nuclei and cell division follows closely the process described,and figured by Wager in Phaseolus and I will not describe it here.

EFFECT OF KI.- Cytoplasm. The cytoplasm becomes granular in appearance even when exposed to 1/2 and 1 percent solutions for only one hour. This is the first and most conspicuous effect upon the cytoplasm. It becomes more and more marked as the series progresses into higher percents and longer periods of treatments. At first the return to normal appearance in the 6 and 24 hour recoveries is quite noticeable but after the severer treatment of 1 1/2 and 2 percents little recovery is made. The rectangular cells bordering the plerome show a very coarsely granular structure in some sections (1 1/2% and 2%) and take the stain so much more readily than the rest that they stand out, under the low power, as a dark line.

The roots treated with 2 percent show a more and more scanty, vacuolated cytoplasm. The cells of the zone of elongation have quite large vacuoles and in some cases the protoplast is drawn slightly away from the cell wall. In the

cells of the zone of differentiation the cytoplasm is reduced to a mere film which stains rather deeply. Death occurs in outer cells of this region before it does in those in the tip, this may be due to the continuence of this influence.

After treatment with 1 1/2 and 2 percent KI, the cytoplasm of cells in the formative and elongating zones contain bodies which from every evidence that I can gather are extruded nuclear matter. I am inclined to believe that this is the case because of the appearance of certain nuclei which seem to be giving off chromatic granules. This will be discussed farther under nuclei. In cells treated with 2 percent KI for 3 hours and longer darkly staining threads appear massed at the end of plerome cells. These resemble the mitochondria described by Mevis (1904).

THE RESTING NUCLEUS - The resting nucleus is the least affected of any of the cell constituents. After more than one hour in 2 percent KI those roots killed at once and after 6 hours show nuclei with irregular outlines. Small protrusions can be seen and it seems quite apparent that granules of chromatic matter, either nucleolar matter or chromatin, are being given off into cytoplasm. In the 24 hour recovery the nuclei are more normal in appearance but the cytoplasm contains roundish masses of material which certainly stain like nuclear matter. I have tried to show these in my drawings, Figs. 1 and 2.

The appearance of two resting nuclei in one cell will be discussed under mitotic figure.

The nucleoli retain their shape and staining reaction

most remarkably. In the higher percents and long periods of treatment they are somewhat reduced in size. The appearance of budding nucleoli is more common in poisoned than in normal roots. The occurrence of nucleoli in what seems to be a state of fission or fusion will be brot up under mitotic figure.

THE MITOTIC FIGURE - One would expect to find so delicate a piece of mechanism as the mitotic figure to be easily affected by the sort of stimulus that affects the cytoplasm so noticeably, but this is not the case. I am unable to state what effect the KI had on the frequency of division for the reason that my root tips were fixed at different times of the day and the periodicity of cell division might mask any effect that was not very marked. I am inclined to believe that the immediate effect is retarding for, tho no counts were made, dividing cells seem more rare in the higher than in the lower percents. This is one of the phases of the problem that I should like to investigate farther.

The persistence of the nucleolus, mentioned in the normal figure, becomes a common feature of division in 1 1/2 percent series. It is less common in lower and higher percents. The nucleolus may be single or multiple, often two are seen situated one at each pole of the spindle.

In treating with 2 percent KI an effect is made on the figure. At first the abnormality is only shown by unequal division of the chromosomes but as the poison is allowed to act for a longer time more radical changes occur. The spindle becomes very irregular with fibres reaching out in all directions

from the equitorial plate, carrying chromosomes to any portion of the cell. Finally the figure becomes a confused mass of fibres and chromatin bodies. (Figs. 3 and 4). Such figures occur in the roots fixed at once after treatment and more rarely in the 6 hour recovery. After 24 hours in water the figures formed are quite normal in outline. The question as to what becomes of the abnormal nuclear divisions is answered, I believe by cells such as shown in figures 5 and 5a,where several small bodies resembling small nuclei appear in a large cell or where small chromatic masses are surrounded by a very irregular nuclear wall. It seems clear that after one of these abnormal divisions the nuclear membrane formed about a small group of chromosomes or about seceral such groups as it would about the daughter nuclei.

One peculiarity of the treated root tips that makes its appearance in the 1 1/2 percent series and becomes more and more common thru the 2 percent series I have left to discuss here. This is the cell with two separate or partially united nuclei. I have shown a series of such cells in outline drawings, figures 6,7, 8 and 9. It is not surprising that such cells should suggest amitotic division or fusion of daughter nuclei depending on which cell one chooses as a start 6 or 9. A figure such as 8 convinces me that neither of these unusual things is really taking place. What has occurred is, the poison has penetrated a cell in the metaphase of division. The kinoplasmic activity has been inhibited by the KI and masses of chromosomes have gone into the resting stage where ever they happened to be. The phragmoplast does not form under influence of the KI as is

evidenced by the great number of cells where nuclear division but no cell division has taken place.

DEATH - The plan of fixing roots after 24 hours of recovery in water was adopted to prevent the study of any cells that were dying, as well as, for the purpose of determining the after effect of the poison. I find that death results in practically all roots after 4 or more hours in 2 percent. The death is slow and does not occur until nearly 24 hours after treatment. Death occurs in the outer cells of the zone of differentiation and in the inner cells of the root cap while other parts of the root are still alive.

CONCLUSIONS.

I do not feel that the problem I took up is solved but rather that the work has progressed to a stage where the possibilities of real investigation along the line are apparent. The conclusions that I might draw at this time could only be tentative, and I prefer to regard them as indications of what will probably be found true.

1. The conclusion that is most firmly established by my study is that the microscopic structure of the cytoplasm is altered by external agents and is an expression of the physiologic state. The change from the finely alveolar structure of the normal to that of a coarsely granular one is noteworthy in the light of the recent discussions as to which of these is the true structure of cytoplasm. This certainly indicates that structure may be now one, then the other, as the physiologic condition of

the cell varies. That this change is not mechanical coagulation by the KI is proved by the facts that it occurs when all other cell structures are uninjured and processes such as cell division go on normally and that return to the usual structure takes place.

2. Cells of different regions respond differently to this stimulus. For example, the cells of the border of the plerome that have been mentioned several times become much more granular than the rest and their nuclei are the first to give off particles of chromatin matter. The narrow plerome cells are the ones that contain the largest number of mitochondria.

3. The KI acts on the cell so as to check normal anabolism or so that the cells are stimulated to activity that requires more than the usual food supply. This may be inferred from the wasting away of the cytoplasm, attended by the formation of vacuoles. As already suggested the death of those cells which are supplied with little reserve cytoplasm (differentiated cells and root cap cells) may be due to starvation because of the effect just mentioned.

4. So far as I have been able to observe the nucleolus does not take such a part as Georgevitch (1908) has described in Lupinus nor as Wager describes in Phaseolus. That is the persisting nucleoli did not appear in any case to be contributing to the body of the chromosomes.

5. A mitotic figure that can become a mass of confused fibres as figured in Figures 3 and 4 cannot be conceived to be made up of a field of force as is maintained by Hartog(1905)

What ever is the nature of the animal spindle the fibres of these spindles must be material.

As I have already stated the chief thing I have gotten from this study is a realization of the possibilities of such a research. I will mention briefly some of the questions that need farther attention.

1. The determination of the chromatin content of the nucleolus and its relation to the formation of the chromosomes would shed much light on the function of the nucleolus.

2. The determination of the extruded chromatic bodies and mitochondria, as to whether they are chromatin or nucleolar matter, would be of theoretical importance. These two studies (1 and 2) could be made thru another staining method, only.

3. The determination as to whether the persistence of the nucleoli in dividing cells results in fibres that stain less deeply or are fewer in number would show whether the nucleoli are kinoplasmic or not.

4. The accelerating or retarding effect of the poison on the rate of cell division is an interesting phase that could be quite easily worked out.

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EXPLANATION OF PLATE.

Figures 1.- Cells showing nuclei giving off nuclear matter.

2 percent KI for two hours.

2.- Nuclear bodies is cells,

34 hours recovery after 2 hours KI.

3. & 4.-

Nuclear figures affected by KI.

3.- 2 percent KI one hour

4.- 2 percent KI two hours.

5. & 5a.-

Recovery from abnormal figures,

6 hour recovery from 2 percent KI for three hours.

6, 7, 8, & 9.-

Outlines of cells showing pseudoamitosis caused by checking mitotic division by KI.

6 and 24 hour recovery after 3 hours KI.

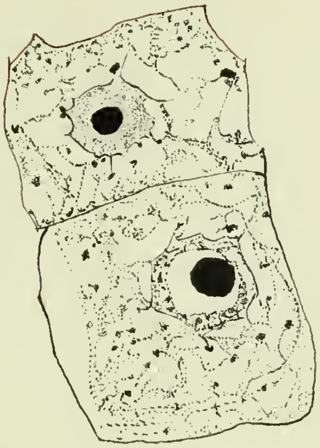

Fig. 1.

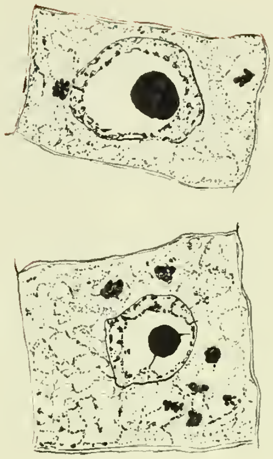

Fig. 2.

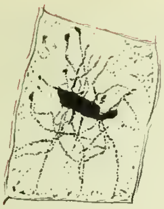

Fig. 3.

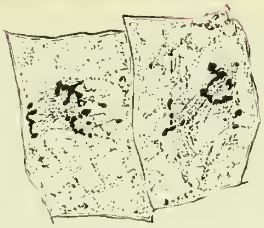

Fig. 4.

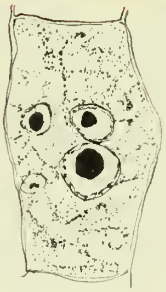

Fig. 5.

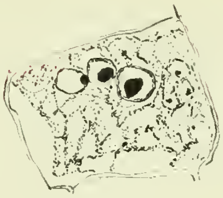

Fig. 5 a.

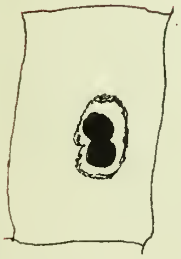

Fig. 6.

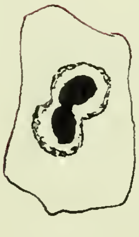

Fig. 7.

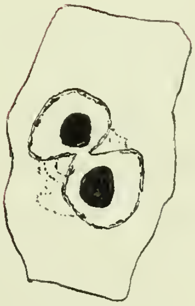

Fig. 8.

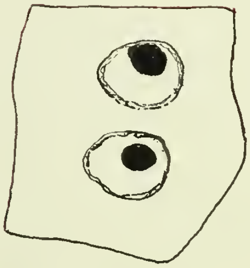

Fig. 9.

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